

Figure S1. The *rfa1* mutants are proficient for intra-chromosomal DSB repair, related to Figure 1

(A) Kinetics of MAT switching for the indicated strains. Errors bars show SD ($n=3$)
 (B) Frequency of DSB-induced direct repeat recombination in the indicated strains. Error bars show SD ($n>4$ for all strains).

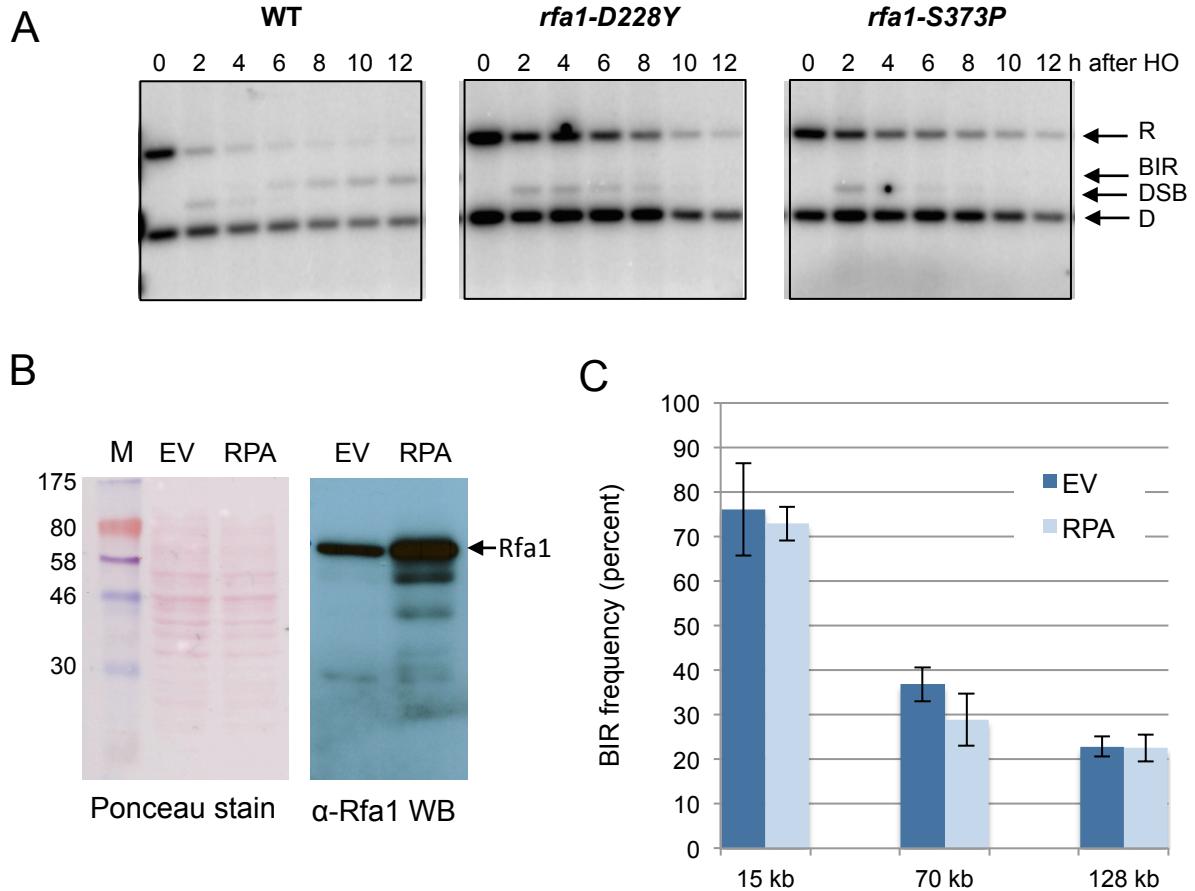


Figure S2. RPA is required, but is not limiting for BIR, related to Figure 2

- (A) Detection of BIR products by EcoV digestion and Southern blot using the 70 kb donor strain (see Fig 2A for schematic of restriction digest pattern).
- (B) Extracts prepared from cells containing pRS425 (EV) or pRS425 expressing all three subunits of the RPA complex.
- (C) BIR frequencies of strains with the donor cassette positioned 15, 70 or 128 kb from the telomere containing empty vector or high copy expression of *RFA1*, *RFA2* and *RFA3*. Error bars show SD from three trials.

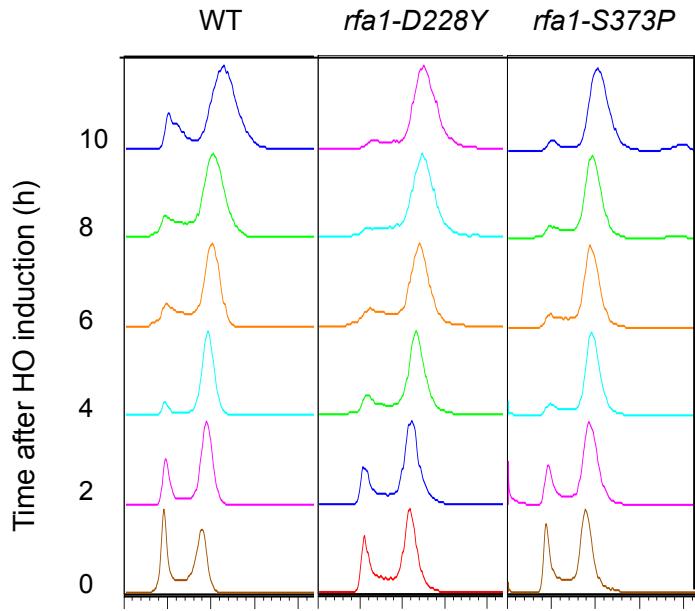


Figure S3. FACS profiles of cells undergoing BIR, related to Figure 2

Cells from the indicated genetic background with the 15 kb BIR donor were fixed at 2 hr time points after HO induction.

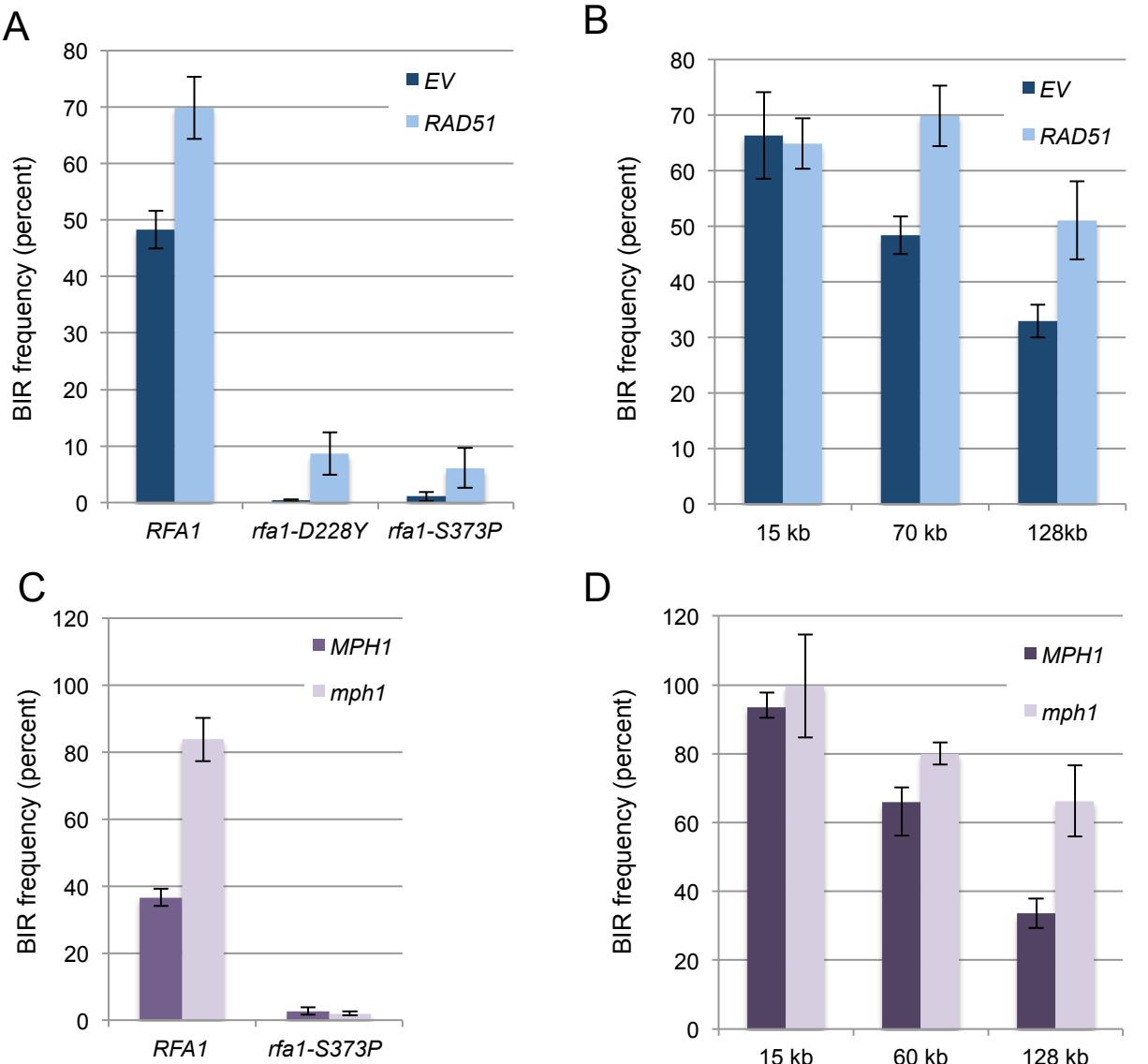


Figure S4. BIR frequency for *RAD51* OE and *mph1* Δ mutants, related to Figure 4

(A) BIR frequency of 70 kb donor strains expressing empty vector (EV) or *RAD51*. Error bars show SD (N=3).

(B) BIR frequency of 15, 70 and 128 kb wild type donor strains expressing EV or *RAD51*. Error bars show SD (n=3).

(C) BIR frequency of the *MPH1* and *mph1* Δ derivatives of the 70 kb donor strains. Error bars show SD (n=3).

(D) BIR frequency of 15, 60 an 128 kb donor *MPH1* and *mph1* Δ derivatives. Error bars show SD (n=3).

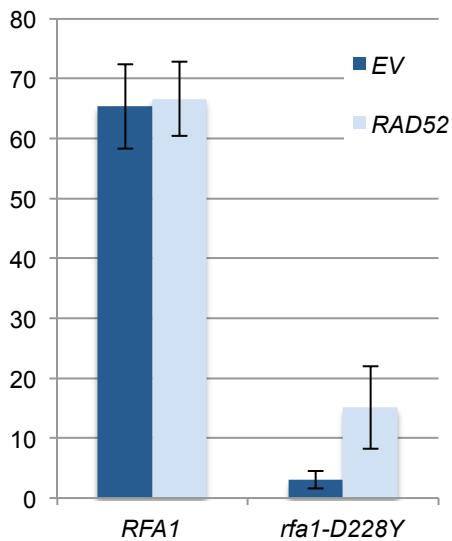


Figure S5. BIR frequency for *RAD52* OE, related to Figure 4

BIR frequency of 15 kb donor strains expressing empty vector (EV) or *RAD52*. Error bars show SD (N=3).

Table S1. Yeast strains, related to Figures 1-5

| Strain | Background | Genotype | Source |
|--------------------------------|------------|--|---------------------------------|
| LSY2714 | W303 | <i>MATa rfa1-S373P</i> | (Deng et al., 2014) |
| LSY2969 | W303 | <i>MATa rfa1-S373P::NatMX4</i> | (Deng et al., 2014) |
| LSY2900-13C | W303 | <i>MATa ade3-GAL-HO</i> | (Deng et al., 2014) |
| LSY2900-5D | W303 | <i>MATa ade3-GAL-HO rfa1-D228Y</i> | (Deng et al., 2014) |
| LSY2900-6D | W303 | <i>MATa ade3-GAL-HO rfa1-D228Y</i> | (Deng et al., 2014) |
| LSY3095-42C | W303 | <i>MATa ade3-GAL-HO rfa1-S373P</i> | This study |
| LSY2680-9A | W303 | <i>MATa-inc lys2::NatMX4 AVT2::lys-HOcs::KanMX6 COS9::TRP1-ys2 (Ch XI 15 kb) ade3::GAL-HO URA3-TK</i> | (Donnianni and Symington, 2013) |
| LSY2752-2.1 | W303 | <i>MATa-inc lys2::NatMX4 AVT2::lys-HOcs::KanMX6 PTK1::TRP1-ys2 (Ch XI 70 kb) ade3::GAL-HO URA3-TK</i> | (Donnianni and Symington, 2013) |
| LSY2767-32C | W303 | <i>MATa-inc lys2::NatMX4 AVT2::lys-HOcs::KanMX6 SYN8::TRP1-ys2 (Ch I 128 kb) ade3::GAL-HO URA3-TK</i> | (Donnianni and Symington, 2013) |
| LSY2710-15A | W303 | <i>MATa-inc lys2::NatMX4 AVT2::lys-HOcs::KanMX6 COS9::TRP1-ys2 (Ch XI 15 kb) ade3::GAL-HO pol32::KanMX6 URA3-TK</i> | (Donnianni and Symington, 2013) |
| LSY2796-12D | W303 | <i>MATa-inc lys2::NatMX4 AVT2::lys-HO_{cs}::KanMX6 ERV46::TRP1-ys2 (Ch I 60 kb donor) ade3::GAL-HO URA3::TK bar1::LEU2</i> | (Donnianni and Symington, 2013) |
| LSY2884-53D | W303 | <i>MATa-inc lys2::NatMX4 AVT2::lys-HOcs::KanMX6 SYN8::TRP1-ys2 (Ch I 128 kb) ade3::GAL-HO URA3-TK mph1::KanMX6</i> | (Stafa et al., 2014) |
| LSY2934-22D | W303 | <i>MATa-inc lys2::NatMX4 AVT2::lys-HO_{cs}::KanMX6 ERV46::TRP1-ys2 (Ch I 60 kb donor) ade3::GAL-HO URA3::TK mph1::KanMX6</i> | This study |
| LSY2948-8A | W303 | <i>MATa-inc lys2::NatMX4 AVT2::lys-HOcs::KanMX6 COS9::TRP1-ys2 (Ch XI 15 kb) ade3::GAL-HO mph1::KanMX6 URA3-TK his3::HphMX4</i> | This study |
| LSY3045-31B, 32C LSY3737-4A | W303 | <i>MATa-inc lys2::NatMX4 AVT2::lys-HOcs::KanMX6 COS9::TRP1-ys2 (Ch XI 15 kb) ade3::GAL-HO rfa1-D228Y URA3-TK</i> | This study |
| LSY3046-3C, 13C | W303 | <i>MATa-inc lys2::NatMX4 AVT2::lys-HOcs::KanMX6 PTK1::TRP1-ys2 (Ch XI 70 kb) ade3::GAL-HO rfa1-D228Y URA3-TK</i> | This study |
| LSY3093-31C | W303 | <i>MATa-inc lys2::NatMX4 AVT2::lys-HOcs::KanMX6 SYN8::TRP1-ys2 (Ch I 128 kb) ade3::GAL-HO rfa1-D228Y</i> | This study |
| LSY3097-15A | W303 | <i>MATa-inc lys2::NatMX4 AVT2::lys-HOcs::KanMX6 COS9::TRP1-ys2 (Ch XI 15 kb) ade3::GAL-HO rfa1-D228Y mph1::KanMX6</i> | This study |
| LSY3208-5B, 5D | W303 | <i>MATa-inc lys2::NatMX4 AVT2::lys-HOcs::KanMX6 PTK1::TRP1-ys2 (Ch XI 70 kb) ade3::GAL-HO rfa1-S373P</i> | This study |

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|--------------------------------|------|---|-------------------------------|
| LSY3224-19A, 26B LSY3713-5B | W303 | <i>MATa-inc lys2::NatMX4 AVT2::lys-HOcs::KanMX6 COS9::TRP1-ys2 (Ch XI 15 kb) ade3::GAL-HO rfa1-S373P</i> | This study |
| LSY3349-3B | W303 | <i>MATa-inc lys2::NatMX4 AVT2::lys-HOcs::KanMX6 COS9::TRP1-ys2 (Ch XI 15 kb) ade3::GAL-HO rfa1-S373P mph1::KanMX6</i> | This study |
| LSY3714-10A, 17B | W303 | <i>MATa-inc lys2::NatMX4 AVT2::lys-HOcs::KanMX6 PTK1::TRP1-ys2 (Ch XI 70 kb) ade3::GAL-HO URA3-TK mph1::KanMX6</i> | This study |
| LSY3714-6B, 13A | W303 | <i>MATa-inc lys2::NatMX4 AVT2::lys-HOcs::KanMX6 PTK1::TRP1-ys2 (Ch XI 70 kb) ade3::GAL-HO URA3-TK mph1::KanMX6 rfa1-S373P</i> | This study |
| LSY2681-1B | W303 | <i>MATa-inc lys2::NatMX4 AVT2::lys-HOcs::KanMX6 COS9::TRP1-ys2 (Ch XI 15 kb) ade3::GAL-HO exo1::HIS3 URA3-TK</i> | This study |
| LSY-2720-12D | W303 | <i>MATa-inc lys2::NatMX4 AVT2::lys-HOcs::KanMX6 COS9::TRP1-ys2 (Ch XI 15 kb) ade3::GAL-HO sgs1D664Δ URA3-TK</i> | This study |
| LSY-2720-6D | W303 | <i>MATa-inc lys2::NatMX4 AVT2::lys-HOcs::KanMX6 COS9::TRP1-ys2 (Ch XI 15 kb) ade3::GAL-HO exo1::HIS3 sgs1D664Δ URA3-TK</i> | This study |
| LSY3713-6C LSY3723-4D | W303 | <i>MATa-inc lys2::NatMX4 AVT2::lys-HOcs::KanMX6 COS9::TRP1-ys2 (Ch XI 15 kb) ade3::GAL-HO rfa1-S373P exo1::HIS3</i> | This study |
| LSY3723-16D, 22A | W303 | <i>MATa-inc lys2::NatMX4 AVT2::lys-HOcs::KanMX6 COS9::TRP1-ys2 (Ch XI 15 kb) ade3::GAL-HO rfa1-S373P sgs1D664Δ</i> | This study |
| LSY3723-25D | W303 | <i>MATa-inc lys2::NatMX4 AVT2::lys-HOcs::KanMX6 COS9::TRP1-ys2 (Ch XI 15 kb) ade3::GAL-HO rfa1-S373P exo1::HIS3 sgs1D664Δ URA3-TK</i> | This study |
| LSY3723-30C | W303 | <i>MATa-inc lys2::NatMX4 AVT2::lys-HOcs::KanMX6 COS9::TRP1-ys2 (Ch XI 15 kb) ade3::GAL-HO rfa1-S373P exo1::HIS3 sgs1D664Δ</i> | This study |
| LSY3737-13C, 14C | W303 | <i>MATa-inc lys2::NatMX4 AVT2::lys-HOcs::KanMX6 COS9::TRP1-ys2 (Ch XI 15 kb) ade3::GAL-HO rfa1-D228Y exo1::HIS3 URA3-TK</i> | This study |
| LSY3737-1D, 5B | W303 | <i>MATa-inc lys2::NatMX4 AVT2::lys-HOcs::KanMX6 COS9::TRP1-ys2 (Ch XI 15 kb) ade3::GAL-HO rfa1-D228Y sgs1D664Δ URA3-TK</i> | This study |
| LSY3737-8C | W303 | <i>MATa-inc lys2::NatMX4 AVT2::lys-HOcs::KanMX6 COS9::TRP1-ys2 (Ch XI 15 kb) ade3::GAL-HO rfa1-D228Y exo1::HIS3 sgs1D664Δ</i> | This study |
| LSY3737-14B | W303 | <i>MATa-inc lys2::NatMX4 AVT2::lys-HOcs::KanMX6 COS9::TRP1-ys2 (Ch XI 15 kb) ade3::GAL-HO rfa1-D228Y exo1::HIS3 sgs1D664Δ URA3-TK</i> | This study |
| LSY3449-62D | W303 | <i>MATa ade2-n::TRP1::ade2-Isce1+ lys2::GAL-Isce1</i> | This study |
| LSY1709-9D | W303 | <i>MATa ade2-n::TRP1::ade2-Isce1+ lys2::GAL-Isce1 rad51::LEU2</i> | (Mimitou and Symington, 2008) |
| LSY3736-6B | W303 | <i>MATa ade2-n::TRP1::ade2-Isce1+ lys2::GAL-Isce1 rfa1-D228Y trp1::KanMX</i> | This study |
| LSY3736- | W303 | <i>MATa ade2-n::TRP1::ade2-Isce1+ lys2::GAL-Isce1 rfa1-</i> | This study |

| 7C | | D228Y | |
|----------------------------|------|---|---------------------|
| LSY3449-48B, LSY3735-3C | W303 | <i>MATα ade2-n::TRP1::ade2-1SceI+ lys2::GAL-1SceI rfa1-S373P</i> | This study |
| YSJ119 | JKM | <i>chromosome III::LEU2 at position (41400) can1::leu2::HOcs</i> | (Jain et al., 2009) |
| LSY3313 | JKM | <i>chromosome III::LEU2 at position (41400) can1::leu2::HOcs rfa1-S373P::NatMX4</i> | This study |
| YSJ130 | JKM | <i>chromosome III::LE-URA3-U2 at position (41400) can1::leu2::HOcs</i> | (Jain et al., 2009) |
| LSY3315 | JKM | <i>chromosome III::LE-URA3-U2 at position (41400) can1::leu2::HOcs rfa1-S373P::NatMX4</i> | This study |
| YSJ131 | JKM | <i>chromosome III::URA3-U2 at position (41400) can1::leu2::HOcs</i> | (Jain et al., 2009) |
| LSY3317 | JKM | <i>chromosome III::URA3-U2 at position (41400) can1::leu2::HOcs rfa1-S373P::NatMX4</i> | This study |
| YSJ133 | JKM | <i>chromosome III::URA3-U2 at position (41400) and LE-HPH (at position 37700) can1::leu2::HOcs</i> | (Jain et al., 2009) |
| LSY3319 | JKM | <i>chromosome III::URA3-U2 at position (41400) and LE-HPH (at position 37700) can1::leu2::HOcs rfa1-S373P::NatMX4</i> | This study |
| YSJ134 | JKM | <i>chromosome III::URA3-U2 at position (41400) and LE-HPH (at position 30800) can1::leu2::HOcs</i> | (Jain et al., 2009) |
| LSY3321 | JKM | <i>chromosome III::URA3-U2 at position (41400) and LE-HPH (at position 30800) can1::leu2::HOcs rfa1-S373P::NatMX4</i> | This study |
| YSJ135 | JKM | <i>chromosome III::URA3-U2 at position (41400) and LE-HPH (at position 24500) can1::leu2::HOcs</i> | (Jain et al., 2009) |
| LSY3323 | JKM | <i>chromosome III::URA3-U2 at position (41400) and LE-HPH (at position 24500) can1::leu2::HOcs rfa1-S373P::NatMX4</i> | This study |

W303 derivatives have the genotype *leu2-3,112 trp1-1 can1-100 ura3-1 his3-11,15 ade2-1*, variations from this genotype are listed above.

All YSJ derivatives have the following genotype: *mata::hisG hoΔ hml::ADE1 hmr::ADE1 leu2::KAN ade3::GAL-HO ade1 lys5 ura3-52 trp1*, differences from this genotype are listed.

References

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